When in endothelial cell culture conditions, the blast cells differentiated to endothelial cells which had the ability to take up Dil-Ac-LDL and formed complex vascular networks in Matrigel. We concluded that: 1) hemangioblast exist transiently in early embryonic development and can form single cell-derived colonies; 2) differentiation of hemangioblasts can be tracked by the use of chosen molecular markers; 3) blast colonies consist of cells having properties of endothelial or hematopoietic precursors, 4) blast cells can be potentially used in regenerative medicine due to their low immunostimulatory potential.

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NASAL EPITHELIAL CELLS OF DONOR ORIGIN AFTER ALLOGENEIC HCT: RESULT OF STEM-CELL PLASTICITY, CELL-FUSION OR TRANSFER OF EPITHELIAL CELL PRECURSORS WITH THE GRAFT?

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Background: Epithelial cells (ECs) are generally believed to originate from epithelial stem cells and not from hematopoietic stem cells (HSCs). However, detection of donor-type myocytes, neurons and skin, liver, lung or intestinal epithelial cells after allogeneic HCT (using XY-FISH and sex-mismatched donor-recipient pairs) has suggested that HSCs may carry a degree of developmental plasticity. This is controversial given artifacts of the XY-FISH or the possibility of hematopoietic-EC cell fusion. Alternatively, donor-type ECs post-transplant could originate from ECs or their precursors transferred with the graft. The goal of this study was to determine whether donor origin nasal ECs exist in HCT recipients, and if so, then the underlying mechanism.

Methods: We collected nasal scrapings and whole blood from 35 HCT survivors, either early (2–3 months, n = 15) or late (4–22 years, n = 20) post-transplant. Bone marrow was grafted in 15 of the 20 late post-transplant patients whereas filgrastim-mobilized blood stem cells were grafted in all 15 early and 5 late post-transplant patients. To avoid the limitations of XY-FISH, chimerism of the ECs was determined as: Nasal cells were stained with cytokeratin (CK) and CD45 antibodies. True ECs (CK+CD45−) were laser captured. DNA extracted from the captured ECs and blood leukocytes was PCR amplified for a panel of 15 autosomal STR markers and an XY-differentiating locus (ABI-Identifiler). In addition, a combination of immunofluorescence staining for CK and FISH for two autosomes was used in 10 patients to assess cell fusion. Epithelial cell precursors in 8 graft specimens were searched by using magnetic separation with CD36 microbeads and staining with CK.

Results: In all 35 HCT survivors, ECs of donor origin were identified accounting for 2.2% to 12.3% (median 6.2 %) of the nasal ECs. The percentage appeared to be ~2-fold higher late posttransplant in marrow compared to blood stem cell recipients and was similar in blood stem cell recipients early and late posttransplant. None of the nasal ECs in 10 HCT survivors exhibited the presence of cell fusion. Epithelial cells/epithelial cell precursors (CD36+CK+) were found in each of the 8 graft specimens.

Conclusion: Donor origin nasal ECs were identified even with a method that obviates the artifacts of XY-FISH. The underlying mechanism appears to be trans-differentiation of HSCs into ECs or the transfer of ECs (or their precursors) with the graft, but not cell fusion.

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ISOLATION AND EXPANSION OF OLIGODENDROCYTES FROM THAWED, CRYOPRESERVED HUMAN UMBLILICAL CORD BLOOD

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While transplantation of unrelated umbilical cord blood (UCB) halts the progression of neurological damage in children with lysosomal storage diseases (LSD), it does not necessarily reverse the damage sustained before transplant. Isolation and expansion of central nervous system progenitor cells from UCB has potential therapeutic application in treating these patients. We recently described the reliable isolation and expansion of oligodendrocyte-like precursor cells (OPC) from freshly collected, non-crypreserved UCB (Tracy et al. Cytotherapy. 2008, 3:1–8). We anticipate utilizing these cells as adjuvant, targeted therapy to reduce the time to donor cell correction/prevention of disease-induced CNS injury. This approach will require use of cyropreserved donor UCB. We now demonstrate the feasibility of isolating and expanding OPCs from a series of thawed, cryopreserved UCB units. Cryopreserved UCB units were thawed using a standard protocol employed in clinical transplantation. Mononuclear cells were isolated by either ficoll density separation or centrifugation after a dextran-albumin wash. Fresh UCB units underwent hetastarch depletion of red blood cells then mononuclear cell isolation by ficoll density gradient separation. Cells were plated at 3 × 10(6) cells/ml in media containing platelet derived growth factor, neurotopin 3, vascular endothelial growth factor, and triiodothyronine. All UCB unit cultures were trypsinized at 21 days, counted, then characterized by flow cytometry after being fixed, permeablized, and labeled with the following antibodies: anti-oligidendrocyte marker 4 (O4), anti-oligidendrocyte marker 1 (O1), anti-myelin basic protein (MBP). To examine phenotypic changes over time, cultures from two units (one thawed, one fresh) were analyzed weekly over 4 weeks. On flow cytometric analysis, 78% of thawed UCB units yielded O4-expressing cells as at least 20% of total events compared with 95% of fresh UCB units.

Table 1: Characteristics of UCB-Derived Oligodendrocytes

	Thawed UCB Units	Fresh UCB Units
Total Units Cultured	27	19
Mean # mononuclear cells plated/UCB Unit	3.03 × 10 (8)	1.53 × 10 (8)
Final oligodendrocyte cell count/UCB Unit	8.41 × 10 (5)	3.41 × 10 (6)
Units with O4 expression >20% of total events	21 (78%)	18 (95%)
Events in gate as % total events	54.06	76.54
O4 expression (% of gated) 85.93 87.88	85.93	87.88
O1 expression (% of gated)	89.84	88.40
MBP expression (% of gated)	28.87	30.51
Co-expression of MBP-O1 (% of gated)	26.67	30.50

Average OPC yield per UCB unit was less for thawed units (8.41 × 10(5)) than fresh (3.41 × 10(6)). However, expression of O1, O4, and MBP was similar. We also noted early expression of the preoligodendrocyte marker O4 by 1–2 weeks in culture, followed by expression of mature oligodendrocyte markers O1 and MBP over 2–4 weeks. Our results demonstrate that oligodendrocyte precursor cells can be derived reliably from thawed, cryopreserved UCB units, and suggest the feasibility of using these cells in human clinical trials.

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ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) FOR RELAPSED, REFRACTORY MYELOID LEUKEMIA AND MDS USING CLOFARABINE (CLO) ± FLUDARABINE (FLU) WITH IV BUSULFAN (BU) AS CONDITIONING THERAPY

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IV Bu/Flu is a safe conditioning regimen, but relapses still occur. We decided to replace Flu with Clo to enhance the regimen antileukemic effect. The immunosuppressive capacity of Clo is unknown, and we gradually replaced Flu with Clo, aimed at retaining safety while evaluating the engraftment-promoting capacity of Clo.

Methods: Patients were randomized to: Arm I: Clo:Flu 10:30 mg/m2, Arm II 20:20 mg/m2, Arm III 30:10 mg/m2; Arm IV Clo 40 mg/m2. The nucleoside analog(s) were/was infused over 1 hour daily ×4 days; Bu was infused over 3 hours (daily AUC of 6,000 mcMol-min ± 10%), immediately after the nucleoside analog(s). 25 pts have been enrolled, 22 are evaluable for engraftment and survival beyond day 100; 16 were males; median age was 46 yrs (6–59). 7 pts had CML (BC: 1, first AP: 3, second AP: 2, and late first CP: 1). 18 patients

had AML: 6 induction failures, 6 in refractory relapse, 3 in untreated relapse, 1 had a second PR, 1 chemo-responsive MDS, 1 in high-risk CR1 [cytogenetics (CG) -7/t(3;12)]. CG were favorable (n = 1), 8 intermediate, and 6 poor prognosis, and in 3 CG were unknown. GVHD-prophylaxis: tacrolimus/mini-MTX, with rabbit-ATG (Thymoglobulin) for unrelated/one Ag-mism. related donor transplants.

Results: 1 patient died of pneumonia (day+77), and 1 of liver GVHD (day+45) as only treatment-related deaths in the 1st 100 days. Main toxicity was mucositis grade 2–3 (50% of the pts). There was no significant hepatic/neurologic toxicity. All 22 evaluable pts engrafted (one progressed by day +30). T-cell chimerism studies at day+30 revealed that groups I+II (lower Clo doses; n = 10) had a median of 87% (17–100) donor (T-cell)-derived DNA, groups III+IV (higher Clo-doses, n = 12) had a median of 100% range (64–100) donor-DNA. By day +100 both cohorts had a median of 100% donor-derived DNA, maintained beyond 6 mo. in all evaluable pts. 3 pts are too early; 10 pts have died: PD (5), GVHD ± infection (4), and pneumonia (1); 1 AML patient is alive after recurrence and 7 AML and 4 CML patients are alive in CR at a median F/up of 9 mos (4–21).

Conclusions: 1) Clo-Bu-based conditioning appears safe in high-risk ML pts. **2)** there should be no concern about the immunosuppressive capability of Clo in this setting **3)** additional studies are warranted to evaluate the antileukemic efficacy of this regimen. Supported by NIH grants CA55164 and CA49639.

SUPPORTIVE CARE

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A RANDOMIZED, CONTROLLED TRIAL OF GRAFT-VERSUS-HOST DISEASE (GVHD) PROPHYLAXIS COMPARING TACROLIMUS AND MYCOPHENOLATE MOFETIL TO TACROLIMUS AND METHOTREXATE: ANALYSIS OF GVHD, RELAPSE AND SURVIVAL

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We conducted a single institution, randomized, controlled trial comparing tacrolimus + MTX (TAC/MTX) to tacrolimus + MMF (TAC/MMF). Eligible patients (pts) were to receive T-replete peripheral blood HCT from 10/10 or 9/10 HLA matched donors. Randomization was stratified based on conditioning regimen intensity. 92 pts were randomized, 45 to TAC/MMF and 47 to TAC/MTX and were all included in the intent-to-treat (ITT) analysis. Two pts were not transplanted and one pt withdrew consent prior to transplant. These pts were excluded in the modified ITT (MITT) analysis. Pts received TAC 0.03 mg/kg/24hr as a continuous IV infusion beginning day -3 with doses adjusted to maintain whole blood levels of 5–15ng/ml. Pts were converted to PO therapy and tapered after 6 months. MTX was given IV at doses of 15mg/m^2 day +1 and 10mg/m^2 on days +3, +6 and +11. MMF was dosed at 15 mg/kg every 12 hours (up to 3g/d) IV beginning day 0, switched to PO and continued for 12 months. Acute GVHD (aGVHD) was graded weekly by standard criteria; chronic GVHD (cGVHD) was scored monthly based on NIH consensus criteria. The groups were balanced with respect to age, diagnosis, disease risk, recipient/donor CMV status, conditioning regimen, donor type and relation. The cumulative incidences of grade 2–4 and 3–4 aGVHD were 79% and 4% in the TAC/MTX arm and 76% and 14% in the TAC/MMF arm (MITT; p = 0.84 and 0.1, respectively). The cumulative incidence of moderate or severe cGVHD at 6 months was 22% in the TAC/MTX arm and 26% in the TAC/MMF arm (MITT; p = 0.88). By ITT analysis, the cumulative incidence of non-relapse mortality suggested an early difference in favor of TAC/MTX, but at 2 years it was 28% for TAC/MTX arm compared to 32% for the TAC/MMF arm (p = 0.41). The cumulative incidence of relapse was 33% in TAC/MTX arm compared to 18% for the TAC/MMF pts (p = 0.06). Overall survival was similar between groups (p = 0.76; 62% TAC/MTX vs. 66% TAC/MMF at 1 year). We conclude that MMF was no better than MTX in preventing GVHD and may perhaps be less effective in preventing more severe forms of aGVHD. Given the direction of effect we observed in severe aGVHD, it is unlikely that a larger trial would show benefit for this endpoint. There was a strong suggestion that relapse was more frequent after MTX than MMF. The beneficial effect of MMF on relapse was offset by the early increase in nonrelapse mortality, so that overall survival was unaffected.

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QUESTIONING THE ROLE OF A NEUTROPENIC DIET IN HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Neutropenic diets (ND) were developed to decrease gut-derived infections and are widely used. After finding that there were no clinical trials supporting their use, we discontinued ND in HSCT in late 2006. Our ND excluded fresh fruits and vegetables as well as black pepper, sushi, deli meats, raw milk products, soft cheeses, raw honey, miso, and yeast. It was replaced by a modified general diet (Non-ND) that permits black pepper, fresh fruits and vegetables, and homemade freshly squeezed juice - but excludes raw tomatoes. The other restrictions remain. 648 consecutive hospitalized patients were studied; 314 on ND and 314 after ND discontinuation (non-ND). All infections occurring during the first hospital stay were analyzed. All patients received ciprofloxacin 500 mg twice a day, fluconazole 200 mg daily (autograft) or voriconazole 200 mg twice daily (allograft), and acyclovir/valacyclovir. The documented infections included in the analysis comprised positive blood, urine (>100,000 organisms), BAL, stool, or wound cultures, and clostridium difficile. As the table shows, there were no differences between

	Neutropenic diet	Non-neutropenic diet	P
Age (years)	57 (18–76)	56 (18–78)	0.89
Male:Female	186:128	190:124	0.97
Allogeneic:Autologous	77:237	72:242	0.64
Diagnosis			0.91
Myeloma	163	165	
NHL	57	64	
AML	54	50	
Other	40	39	
Conditioning regimen			0.97
High-dose Melphalan	163	165	
BEAM	45	42	
Busulfan-fludarabine	32	33	
Reduced-intensity	33	36	
Median days to neutrophil recovery	12	12	0.91
Positive cultures during neutropenia	97	89	0.49
CoNS/MSSA/MRSA + miscellaneous gram-positive	32	33	1
C difficile	24	22	0.88
E faecium (VRE)	9	9	1
Non-VRE Enterococci	3	3	1
Gram-negative bacilli	15	9	0.21
S viridans	11	10	1
Fungus	4	3	1
Positive cultures after resolution of neutropenia	48	20	<0.001
CoNS/MSSA/MRSA + miscellaneous gram-positive	21	9	0.028
C difficile	9	2	0.069
E faecium (VRE)	7	3	0.34
Gram-negative bacilli	8	4	0.38
S viridans	2	1	1
Fungus	1	1	1
VRE surveillance culture positivity acquired during hospitalization	56	31	<0.004